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Salinity Tolerance of Taif Roses by Gibberellic Acid (GA_3)

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Abstract: ***In order to study the effects of salinity on rose and the alleviation of its effects by GA_3, different salinity concentrations i.e. 0, 1, 2 and 4 dSm^{-1} NaCl and GA_3 at 0, 50 and 100 mgL^{-1} on growth and some physiological as well as biochemical and mineral content were investigated. Salinity treatments significantly decreased plant height, branch number and both leaf and stem dry weights compared with the control. Salinity treatments also reduced leaf area and relative water content (RWC), however the stomatal density was increased. Leaf chlorophyll content, N, P, K, Ca and Mg were reduced with increasing salinity concentrations. Meanwhile, Na, Cl and total soluble sugars were gradually increased with increasing salinity concentration. Membrane permeability, proline accumulation and the antioxidant enzymes activities (SOD, CA and POD) of rose leaves were increased by salinity. GA_3 treatments alleviated the negative effects of salinity on the growth and physiological and biochemical parameters previously mentioned. The obtained results suggest that GA_3 play an important role in the defense system against salinity in rose plant through increasing the antioxidant enzyme activities and proline content as well as preventing ion homeostasis.***

Keywords: salinity; GA_3; roses; antioxidant enzymes; membrane permeability; proline; nutrients

1. Introduction

Rose (*Rosa damascina* var. *trigintipetala*) is an economical aromatic plant which cultivated for obtaining volatile oil. There are several pharmacological properties of rose including antioxidant, anti-HIV, anti-bacterial, hypnotic, anti-diabetic, and relaxant effect on tracheal chains have (**Boskabady et al., 2011**). Moreover, it used as ornamental plants in parks, gardens, and houses and they are principally cultivated for using in perfume, medicine and food industry (**Jabbarzadeh and Khosh-Khui, 2005**). Rose water was scattered at weddings to ensure a happy marriage and are symbol of love and purity and are also used to aid meditation and prayer. Because of the low oil content in *R. damascena* and the lack of natural and synthetic substitutes, essential rose oil of rose consider one of the most expensive ones in the world markets (**Baydar and Baydar, 2005**).

There are several factors affecting rose growth and productivity including salinity. Roses are generally sensitive to salinity that exceeds 3.0 dSm^{-1}, but some cultivars can tolerate up to 3.5 dSm^{-1}without reduction in yield and quality (**Cabrera, 2003**). Roses have been classified as very poor salinity tolerance and the growth was reduced by 25 - 50 % when plants exposed to 2 - 3 dSm^{-1} salinity level (**Bernstein et al., 1972**). Despite the fact that they were developed under crop management conditions that are being phased out and with cultivars that are now obsolete (**Cabrera and Perdomo, 2003**), the reference thresholds to salinity conditions for roses are still in use today. Increasing salinity levels significantly decreased shoot dry weight as well as rose flower number (**Caia et al., 2014).**

Salinity stress is a major contributor in decreasing the crop productivity and threatening the agricultural sustainability (**Mckee et al., 2004**). Salinization is rapidly increasing on a global scale and currently affects more than 10 % of arable land, which results in a decline of the average yields of major crops greater than 50% (**Wang et al., 2009**). Soil salinity is a major constraint to common economic crops in many arid and semi-arid regions of the world, which affects plants through osmotic, specific ion and oxidative stresses (**Pitman and Läuchli, 2002**). Salinity stress limits plant development by adversely affecting various biochemical reactions and physiological processes such as photosynthesis, antioxidant metabolism, mineral nutrients homeostasis, osmolytes accumulation and hormonal signaling (**Khan et al., 2012**).

It has been reported that the effects of salinity are generally summarized as water stress, salt stress and stress due to ionic imbalance (**Tunctürk et al., 2011**). Sodium chloride (NaCl) is the most commonly encountered source of salinity (**Li et al., 2006**). Exposure of plants to extreme conditions such as high salinity causes a diverse set of physiological, morphological and developmental changes (**Jampeetong and Brix, 2009**). Salinity adversely affected the vegetative growth characteristics and dry weight (**Shoresh et al., 2011**) and leaf area (**Khalid and Cai, 2011**). Salinity stress also affected some physiological parameters such as chlorophyll content, total soluble sugars and proline content. In this concern, several authors indicated that the chlorophyll content was significantly decreased as a result of salt stress (**Tuna et al., 2008; Khalid and Cai, 2011; Shoresh et al., 2011; Celik and Atak, 2012**). On the other hand, salt stress affected proline content and total soluble sugars in an opposite manner.

The accumulation of proline in leaves (**Eraslan et al., 2007; Tuna et al., 2008; Celik and Atak, 2012**) and total soluble

sugars (**Khalid and Cai, 2011**) have been reported under salt stress. Salinity stress induces over production of reactive oxygen species (ROS) (**Nazar et al., 2011; Khan et al., 2012)** that triggers lipid peroxidation, DNA damage, inhibition of photosynthesis and disturbance in mineral nutrient status (**Nazar et al., 2011; Turan and Tripathy, 2012**). The generation of ROS is limited or scavenged by an antioxidant system including antioxidant compounds and antioxidant enzymes (**Foyer and Noctor, 2003).**

Recently, investigations have focused more on the mechanisms of salt tolerance in plants (**Munns and Tester, 2008**). Phytohormones have been shown to influence salinity tolerance through modulating several physiological processes and biochemical mechanisms (**Fatma et al., 2013**). Their role in salinity stress is critical in modulating physiological responses that lead to adaptation of plants to an unfavorable environment. Among them, the role of GA_3 in stress tolerance and enhancing growth under saline conditions has been reported (**Iqbal et al., 2011**). Reports concerning the foliar application of GA_3 under saline conditions are scarce. A few studies; however, pinpointed the ability of its foliar pre-treatment to overcome adversities of NaCl (**Chakraborti and Mukherji, 2003**) as it alleviates the pessimistic effects on pigment contents and water use efficiency (**Aldesuquy and Ibrahim, 2001**). GA_3 treatment alleviated the negative effects of salinity on the morphological traits and physiological attributes such as chlorophyll content, stomatal conductance and transpiration rate **(Misratia et al, 2013)**. The exogenous application of GA_3 on seedling growth under salt stress conditions provides an attractive approach to encounter the effects of salinity (**Liopa-Tsakalidi and Barouchas, 2011**). The exogenous application of GA_3 produced some benefit in alleviating the adverse effects of salt stress and also improved growth, development and yield (**Javid et al., 2011; Shaddad et al., 2013**). To date, reports concerning the salinity tolerance of *Rosa damascina* var. *trigintipetala* and ability of GA_3 to alleviate the negative effects of salinity are scarce. Therefore, the aim of this study was to investigate the effects of salt stress on the growth and some physiological responses i.e. chlorophyll content, total soluble sugars, proline content, mineral accumulation and antioxidant enzyme activity in Taif roses. In addition, the alleviatory effects of salinity by GA_3 on the previous characters were also investigated.

2. Materials and Methods

2.1 Plant Materials and Treatments

A pot experiment was conducted at the greenhouse of Biology Department, Faculty of Science, Taif University, Saudi Arabia during 2013 and 2014 seasons to investigate the salinity tolerance of *Rosa damascina* var. *trigintipetala* and the ability of GA_3 to alleviate the negative effects of salinity. The salinity levels applied in this experiment were 0, 2, 3 and 4 dSm^{-1} and GA_3 concentrations were 0, 50 and 100 mg L^{-1}. The treatments were arranged in a complete randomized block (split-plot) design with four replicates. The main plots comprehend the salinity treatments while GA_3 treatments were in the sub-plots. The physical and chemical characteristics of the soil used in this study were (sand, 81.30 %, silt 6.80 % and clay 11.90 %) and chemical properties were (pH, 8.08, EC, 2.11 dsm^{-1}, OM, 0.14 %, Total $CaCO_3$, 0.84 %, Na^+, 3.42 ($meqL^{-1}$), Ca^{+2}, 43.12 ($meqL^{-1}$), SO_4^{-2}, 48.51 ($meqL^{-1}$), HCO_3, 2.06 ($meqL^{-1}$), Cl^-, 0.62 ($meqL^{-1}$), total N^+, PO_4^{-3}, K^+ were 0.18, 0.039 and 0.049 %, respectively).

2.2 Growth Characters

Data concerning plant height (cm), branch number/plant, leaf and stem dry weights (g) and leaf area (cm^2) were recorded. In order to determine leaf area, blade area was measured using digital image analysis according to the method of **Matthew et al. (2002).** Digital image of the leaf blade was created in digital format using a Hewlett- Packard scanner (Hewlett Packard, Cupertino, ca), image was scanned at dot per inch (100 dpi), the blade area was measured using public domain software (scion image version 4.02).

2.3 Stomatal Density

To measure stomatal density, two mid-level leaves of each branch were chosen. The abaxial and adaxial faces of each leaf were covered with dental silicon (Silicon CyF, Herpo Productos Dentarios Ltda., Santiago). After solidification, the silicon strips were peeled off and colorless fingernail polish was applied to the molds. The resulting images of the leaf surfaces were observed with a light microscope. Two to four counts were made in each sample for this parameter. The stomatal density was obtained using a Nikon Labophot-2 microscope with a Leitz Wetzlar 12.5 x graduated ocular **(Botti et al., 1998)**.

2.4 Leaf relative water content (%)

Leaf RWC was determined and calculated from the following relationship:
$(W_{fresh} - W_{dry}) / (W_{turgid} - W_{dry})$ x 100, where W_{fresh} is the sample fresh weight, W_{turgid} is the sample turgid weight after saturating with distilled water for 24 h at 4 °C, and W_{dry} is the oven-dry (70 °C for 48 h) weight of the sample (**Weatherley, 1950**).

2.5 Membrane Permeability (%)

Membrane permeability of the excised leaves was measured at the end of the experiment (**Yan et al., 1996**). Fresh part from the middle of leaves was weighed into a glass beaker containing reverse osmosis water. The beakers were immersed at 30 ± 1˚C for 3 h, and then the conductivity of the solution was measured with a conductivity meter. The conductivity was measured again after boiling the samples for 2 min when the solution was cooled to room temperature. The percentage of electrolyte leakage was calculated by using the equation, EC % = (C_1/C_2) X 100, since C_1 and C_2 are the electrolyte conductivities measured before and after boiling, respectively.

2.6 Antioxidant Enzyme Activities

To obtain the enzyme extract for antioxidant enzymes determination, the method previously described by **Hassan and Mahfouz (2012)** was used. The resulting supernatant

was used as an enzyme extract to determine superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities. Soluble protein contents of the enzyme extract were assayed according to the method **of Bradford (1976).**

SOD (Ec 1.15.1.1) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). SOD activity was expressed as SOD units min^{-1} mg^{-1} protein. One unit of SOD was considered to be the amount of enzyme required to inhibit NBT reduction by 50 % as described by **Giannopolitis and Ries (1977)** by measuring the absorbance at 560 nm by a spectrophotometer (type GBC, UV/VIS 916).

CAT (Ec 1.11.1.6) activity was spectrophotometrically estimated by method of **Clairbone (1985),** following the disappearance of H_2O_2 at 240 nm. The level of enzyme activity was expressed as µ mol min^{-1} mg^{-1} protein.

POD (Ec 1.11.1.7) activity was tested according to **Shanon et al. (1966).** Sodium acetate buffer (0.1 M) and 0.5 % guaiacol were added to the enzyme extract. The reaction was started with 0.1 % H_2O_2. The rate of change in absorbance was spectrophotometrically measured at 470 nm and the level of enzyme activity was expressed as µ mol min^{-1} mg^{-1} protein.

2.7 Proline Content (µmol g^{-1} FW**).**

The free proline content was determined as described by **Bates et al. (1973).** Frozen leaf tissue (0.5 g) was homogenized with 10 mL of 3 % sulfosalicylic acid at 4 °C. Then, the obtained extract was filtered with Whatman No. 2. Mixture of 2 mL of filtrate, 2 mL of acid-ninhydrin, and 2 mL of glacial acetic acid were mixed in a test tube and incubated at 100 °C for 1 h. The reaction was terminated on ice, and the reaction mixture was then extracted with 4 mL of toluene. The chromophore-containing toluene was separated from the hydrated phase. The absorbance at 520 nm was spectrophotometrically determined with toluene as the blank. The proline concentration was calculated based on a standard curve and was expressed as µmol g^{-1} FW.

2.8 Chemical Analysis

2.8.1. Chlorophyll content
Randomly samples of fresh leaves were taken from the middle part of stem for chlorophyll determination. Chlorophyll content was determined according to **Sadasivam and Manickam (1992)** by using spectrophotometer (Pharmacia, LKB-Novaspec II and calculated as (mgg^{-1} FW).

2.8.2. Total Soluble Sugars (TSS %)

Total soluble sugars will determine in leaf samples according to the method of **Dubois et al., (1956)**.

2.8.3. Nutrient elements
Nitrogen, phosphorus, potassium, calcium, magnesium, sodium and chloride were determined as described in **A.O.A.C. (1995).**

2.9. Statistical analysis

The results of two experiments were pooled (*n=8*) and the analysis of variance (ANOVA) was performed using MSTAT program, USA. Means were separated using LSD test at a significance level of 0.05.

3. Results

3.1 Growth Parameters
Increasing salinity level from 0 to 4 dSm^{-1} resulted in a gradual decrease in plant height, branch number per plant, leaf and stem dry weights and leaf area (Tables 1 and 2). However, foliar application with GA_3 alleviated the negative effects of salinity since the above mentioned characters were significantly increased. The most pronounced promotion was observed with the highest GA_3 level.

3.2 Relative Water Content (RWC)

The relative water content of rose leaves was decreased as a result of applying different salinity levels. On the other hand, applying GA_3 whether at 50 or 100 mg L^{-1} resulted in a significant increase in RWC relative to the control (Table 3). The RWC was 88, 81, 72.67 and 66.67 % when salinity was applied at 0, 2, 3 and 4 dS m^{-1} while it was 93.33, 87, 78.33 and 74 % when GA_3 was used at 100 mg L^{-1} under the same salinity levels, respectively.

Table 1: Alleviatory effects of salt stress by gibberellic acid on plant height and branch number/plant of Taif`s rose plants

Treatments	GA_3 concentrations (mg L^{-1})							
	0	*50*	*100*	*Mean*	*0*	*50*	*100*	*Mean*
Salinity levels (dsm^{-1})	Plant height (cm)				Branch number/plant			
0	49.89	53.71	59.47	54.35	4.68	4.9	5.43	5
2	47.06	51.12	58.48	52.22	3.22	4.96	5.4	4.53
3	41.87	47.69	55.12	48.22	3.52	4.42	5.1	4.35
4	36.34	42.09	51.63	43.35	2.62	3.74	4.29	3.55
Mean	43.79	48.65	56.18		3.51	4.51	5.06	
LSD 0.05								
Salinity	2.47				0.67			
GA_3	2.15				0.53			
Salinity x GA_3	3.86				0.92			

3.3 Stomatal Density

Data shown in Table (3) clearly empathized that stomatal density in rose leaves were increased due to salinity treatments. However, GA_3 treatments did not affect the stomatal density in this experiment. The highest stomatal density (95.67) was recorded by the treatment of 4 dSm^{-1} compared with (71.67) which obtained by the untreated control.

Table 2: Alleviatory effects of salt stress by gibberellic acid on leaf dry weight (g), stem dry weight (g) and relative water content (RWC) of Taif's rose plants

Treatments	GA_3 concentrations (mg L^{-1})											
	0	50	100	Mean	0	50	100	Mean	0	50	100	Mean
Salinity levels (dsm^{-1})	Leaf dry weight (%)				Stem dry weight (%)				RWC (%)			
0	26.22	28.18	29.45	27.95	48.62	49.13	51.25	49.68	90	90.33	93.33	90.55
2	25.45	27.19	28.52	27.05	46.72	47.15	49.22	47.7	81	84	87	84
3	21.38	23.7	25.62	23.57	44.54	46.37	48.23	46.38	72.67	72.33	78.33	75.44
4	19.21	22.65	24.18	22.01	41.17	43.68	46.74	43.86	66.67	71.67	74	70.78
Mean	23.07	25.43	26.94		45.26	46.58	48.86		90	90.33	93.33	
LSD 0.05												
Salinity	1.38				2.43				3.42			
GA_3	1.25				2.17				3.35			
Salinity x GA_3	1.64				3.21				4.08			

3.4 Chlorophyll Content

Data presented in Table (3) show that chlorophyll content of rose leaves was significantly as well as gradually decreased with increasing salinity levels from 0 to 4 dSm^{-1}. However, the chlorophyll content was gradually increased with increasing GA_3 concentrations from 0 to 100 mg L^{-1}. The chlorophyll decreased occurred by salinity was alleviated when GA_3 was applied more so with the treatment of 100 mg L^{-1}.

3.5 Total soluble sugars (TSS %)

It could be noticed from data in Table (4) that increasing salinity levels from 0 to 4 dS m^{-1} significantly increased TSS and the same trend was observed when GA_3 was applied at 50 or 100 mg L^{-1}. This increment was clearly appeared when the combination between salinity and GA_3 was occurred. The TSS was (8.47, 9.44, 10.67 and 12.50 %) for the treatments of (0, 2, 3 and 4 dSm^{-1}) while it was (9.73, 10.11, 12.44 and 14.53 %) when GA_3 at 100 mg L^{-1} was used under the same salinity levels.

Table 3: Alleviatory effects of salt stress by gibberellic acid on leaf area (cm^2) and stomatal density (No. mm^2) of Taif's rose plants

Treatments	GA_3 concentrations (mg L^{-1})							
	0	*50*	*100*	*Mean*	*0*	*50*	*100*	*Mean*
Salinity levels (dsm^{-1})	Leaf area (cm^2)				Stomatal density (No/mm^2)			
0	5.75	6.54	7.55	6.58	71.67	72	72.67	72.11
2	4.58	5.29	6.72	5.53	79.33	79.67	79.67	79.56
3	3.73	4.82	5.24	4.6	85	85.33	84.67	85
4	3.14	3.92	4.32	3.79	95.67	95	94.33	95
Mean	4.3	5.14	5.96		82.92	83	82.84	
LSD 0.05								
Salinity	0.67				2.85			
GA_3	0.62				2.46			
Salinity x GA_3	0.74				2.24			

3.6 Proline content (µmol g^{-1} FW)

The proline content of rose leaves treated with salinity or GA_3 was gradually increased with increasing the level of both of them. The promotion effect of salinity on proline accumulation was enhanced when salinity combined with GA_3 treatments. The highest proline content (6.58 µmol g^{-1} FW) was recorded by the treatment of 4 dSm^{-1} salinity combined with GA_3 at 100 mg L^{-1} (Table 4).

Table 4: Alleviatory effects of salt stress by gibberellic acid on chlorophyll content (mg^{-1} FW) and total soluble sugars (TSS %) of Taif's rose plants

Treatments	GA_3 concentrations (mg L^{-1})							
	0	*50*	*100*	*Mean*	*0*	*50*	*100*	*Mean*
Salinity levels (dsm^{-1})	Chlorophyll content (mg^{-1} FW)				TSS (%)			
0	.96	1.08	1.22	1.09	8.47	8.96	9.73	9.05
2	.88	.95	.86	.93	9.44	9.87	10.11	9.81
3	.76	.81	.83	.80	10.67	11.75	12.44	11.62
4	.72	.76	.78	.76	12.50	13.33	14.53	13.45
Mean	.83	.90	.92		10.27	10.98	11.70	
LSD 0.05								
Salinity	.08				.92			
GA_3	.06				.86			
Salinity x GA_3	1.01				1.08			

3.7 Membrane Permeability (%)

The effects of salinity levels and GA_3 concentrations on membrane permeability were presented in Table (4). The membrane permeability was increased with increasing salinity levels and reached its maximum value by applying the highest salinity level (4 dS m^{-1}). On the other hand, GA_3 treatments reduced the membrane permeability whether applied alone or combined with salinity levels. GA_3 treatment alleviated the negative effect of salinity on membrane permeability and the highest GA_3 level recorded the best results in this concern under any salinity level.

3.8 Antioxidant Enzyme Activity

Data presented in Table (5) indicate that both salinity and GA_3 treatments increased the activity of CAT, SOD and POD enzymes compared with the control. The highest activities were obtained by applying the highest salinity (4 dSm^{-1}) and GA_3 (100 mg L^{-1}) levels. The combined treatments between salinity and GA_3 had greater effect in this concern compared with each of them alone.

Table 5: Alleviatory effects of salt stress by gibberellic acid on proline and membrane permeability of Taif's rose plants

Treatments	GA_3 concentrations (mg L^{-1})							
	0	*50*	*100*	*Mean*	*0*	*50*	*100*	*Mean*
Salinity levels (dsm^{-1})	Proline				Membrane permeability			
0	1.39	1.64	1.95	1.66	7.82	6.81	5.14	6.59
2	2.11	2.89	3.74	2.91	9.42	7.82	6.15	7.80
3	2.16	3.50	5.19	3.62	11.73	10.22	8.72	10.22
4	2.97	4.63	6.58	4.67	13.83	10.74	9.22	11.27
Mean	2.11	3.17	4.37		10.70	8.90	7.31	
LSD 0.05								
Salinity	.74				.84			
GA_3	.42				.76			
Salinity x GA_3	.58				.98			

3.9 Nutrient Elements

Macro and micro elements were affected by salinity as well as GA_3 treatments as shown in Tables (6, 7, 8 and 9). It could be noticed that N, P, K, Ca and Mg had the same trend since their contents decreased with increasing salinity levels however GA_3 treatments increased these elements in rose leaves. GA_3 application especially at highest level (100 mg L^{-1}) alleviated the negative effects of salinity and increased the absorption of the previous elements. Concerning Na and Cl concentrations, increasing salinity levels led to a gradual increase in their levels and the highest values were obtained by applying 4 dSm^{-1} treatment. On the other hand, foliar application of GA_3 at 50 or 100 mg L^{-1} reduced the concentration of both elements in rose levees and the reduction was more pronounced when the highest level of GA_3 was used.

Table 6: Alleviatory effects of salt stress by gibberellic acid on antioxidant enzyme activities (CAT, POX and SOD) of Taif's rose plants

Treatments	GA_3 concentrations (mg L^{-1})											
	0	50	100	Mean	0	50	100	Mean	0	50	100	Mean
Salinity levels (dsm^{-1})	CAT				POX				SOD			
0	.98	1.10	1.24	1.07	18.33	22.67	26.67	22.56	.46	.43	0.48	0.47
2	1.16	1.35	1.57	1.33	22.33	25.67	30.33	26.11	.53	.64	0.74	0.64
3	1.85	1.98	2.12	1.97	28.67	32.67	36.00	32.44	.64	.78	0.93	0.78
4	2.11	2.32	2.49	2.35	36.67	40.33	46.67	41.22	.81	.97	1.20	0.99
Mean	1.53	1.86	1.68		26.50	30.34	34.92		.61	.72	0.84	
LSD 0.05												
Salinity	.19				1.27				.08			
GA_3	0.17				1.11				.07			
Salinity x GA_3	0.22				1.58				1.06			

Table 7: Alleviatory effects of salt stress by gibberellic acid on leaf mineral contents (N, P and K %) of Taif's rose plants

Treatments	GA_3 concentrations (mg L^{-1})											
	0	50	100	Mean	0	50	100	Mean	0	50	100	Mean
Salinity levels (dsm^{-}	N				P				K^{+}			
0	2.17	2.25	2.37	2.27	0.47	0.48	0.50	0.48	2.34	2.41	2.54	2.43
2	1.91	2.13	2.27	2.11	0.43	0.46	0.47	0.45	2.14	2.22	2.29	2.21
3	1.79	1.93	2.09	1.94	0.40	0.43	0.46	0.43	1.84	2.08	2.20	2.04
4	1.67	1.84	1.99	1.83	0.38	0.40	0.44	0.41	1.62	1.73	1.92	1.76
Mean	1.89	2.04	2.18		0.42	0.44	0.47		1.99	2.11	2.24	
LSD 0.05												
Salinity												
GA_3												
Salinity x GA_3												

Table 8: Alleviatory effects of salt stress by gibberellic acid on leaf mineral contents (Ca^{+2} and Mg^{+2}) of Taif's rose plants

Treatments	GA_3 concentrations (mg L^{-1})							
	0	50	100	Mean	0	50	100	Mean
Salinity levels (dsm^{-1})	Ca^{+2}				Mg^{+2}			
0	16.38	16.65	16.85	16.63	0.76	0.78	0.82	0.78
2	15.05	16.1	16.12	15.76	0.68	0.72	0.75	0.72
3	13.73	14.82	15.64	14.73	0.62	0.65	0.73	0.67
4	12.11	13.86	14.92	13.63	0.54	0.58	0.69	0.6
Mean	14.32	15.36	15.88		0.65	0.68	0.75	
LSD 0.05								
Salinity	1.18				0.07			
GA_3	1.14				0.05			
Salinity x GA_3	1.86				0.09			

Table 9: Alleviatory effects of salt stress by gibberellic acid on leaf mineral contents (Na^{+} and Cl^{-}) of Taif's rose plants

Treatments	GA_3 concentrations (mg L^{-1})							
	0	*50*	*100*	*Mean*	*0*	*50*	*100*	*Mean*
Salinity levels (dsm^{-1})	Na^{+}				Cl^{-}			
0	0.27	0.27	0.23	0.26	6.51	6.32	5.93	6.26
2	0.43	0.31	0.29	0.34	9.33	8.56	7.61	8.5
3	0.98	0.48	0.32	0.59	11.61	9.87	4.85	9.98
4	1.21	0.67	0.54	0.81	13.75	10.82	9.57	11.38
Mean	0.72	0.43	0.35		10.3	8.89	6.99	
LSD 0.05								
Salinity	0.08				0.84			
GA_3	0.05				0.68			
Salinity x GA_3	1.03				1.17			

4. Discussion

The obtained results showed that the vegetative growth of rose plant was negatively affected by salinity treatments. The reduction of growth is a common indicator of salt stress because of inadequate water uptake (**Borsani et al., 2003**). The plant height, branch number, leaf and stem dry weights as well as leaf area were gradually decreased with increasing salinity levels. The vegetative growth reduction occurred as a result of salinity may be due to the reduction of both cell division and enlargement (**Yasseen et al., 1987**). Otherwise, inhibition of shoot growth has been considered a whole plant adaptation to salt stress (**Qaderi et al. 2006**). The suppression of growth under salt-stress may be also due to direct effects of ion toxicity especially Na and Cl or indirect effects of saline ions that cause soil/plant osmotic imbalance (**Hajiboland et al., 2010**). **Caia et al. (2014)** reported that under salt stress the uptake of water and some mineral nutrients were restricted and hence plant growth and development were inhibited, as well as a series of metabolic functions. These results support the others obtained by (**Shoresh et al., 2011; Khalid and Cai, 2011**).

Decreasing RWC in rose leaves as our data indicated may be a possible explanation for decreasing the growth parameters because it considers as an important parameter for water statues. As a result of a reduction in water content under salt stress a loss of turgor was occurred and resulted in limited water availability for the cell extension process (**Katerji et al., 1997**). These results support the previous results obtained by **Tuna et al. (2008)** and **Ali et al. (2012).**

Increasing stomatal density by salinity may be occurred to make an adaptation to salt and inhabitation of its uptake. Stress has been found correlate with increased stomatal density (**Clifford et al., 1995;Heckenbergar et al., 1998;Pääkkönen et al., 1998**). Salinity treatments also decreased the chlorophyll content of rose leaves. This decrease may be due to a reduction in the uptake of minerals i.e. Mg needed for chlorophyll biosynthesis (**Sheng et al., 2008**), membrane deterioration (**Ashraf and Bhatti, 2000**), or the suppression of specific enzymes that are responsible for the synthesis of photosynthetic pigments (**Murkute et al., 2006**). Our results support the first two reasons because we observed a significant decrease in Mg and increase in membrane permeability under salt stress. Decreasing total chlorophyll content of leaves by increasing salinity has been previously reported (**Tuna et al., 2008; Shoresh et al., 2011; Celik and Atak, 2012**).

A significant increase of total soluble sugars in salt stressed plants was observed. This increment may be occurred to regulate the osmotic potential under salinity treatments (**Teixeira and Pereira, 2007**) or to sustain metabolism, prolong energy supply and for better recovery after stress relieve (**Slama et al., 2007**). These results are in accordance with the others of **Khalid and Cai (2011)** who revealed that salinity stress increased the activity of sucrose phosphate synthase; the key enzyme in the sucrose synthesis pathway, consequently, the total soluble sugars was increased. The accumulation of compatible compounds (osmolytes) including proline is related to improvement of plant tolerance to salt because of its ability to overcome osmotic and water stress and maintain nutrients homeostasis and ion compartmentalization (**Nazar et al., 2011; Khan et al., 2012**). Proline acts as a compatible osmolyte, enzyme protectant, free radical scavenger, cell redox balancer, cytosolic pH buffer and stabilizer for subcellular structures and therefore, it plays a protective function against salinity stress in plants (**Verbruggen and Hermans, 2008**). Such proline accumulation as a result of salt stress is well documented (**Celik and Atak, 2012; Ashfaque et al., 2014)**.

The membrane permeability of rose leaves was significantly increased as a result of salinity application compared with the control. These results could be explained through the negative effects of salinity on Ca level since Ca is required to improve membrane stability (**Shoresh et al., 2011**). Our results show that the antioxidant enzymes activities (CA, SOD and POD) were increased by salt stress compared to the control. There is evidence that salt stress can induce oxidative stress due to generation of reactive oxygen species (ROS) (**Gill and Tuteja, 2010**). Under salinity stress which considers as an oxidative stress, plants produce (ROS), which are harmful to plant growth due to their detrimental effects on the sub cellular components and metabolism of the plant, leading to the oxidative destruction of cells and finally cause deterioration of membrane lipids, leading to increased leakage of solutes from membranes (**Mishra and Choudhuri, 1999**). As a result of ROS production, plant cell has to activate the antioxidant defense system including enzymatic antioxidant to scavenge ROS (**Sairam et al. 2005**). It has been reported that that high peroxidase activity is correlated with the reduction of plant growth and this increment may play an important role as defense against salt stress (**Agarwal and Pandey, 2004**). The increment in antioxidant enzyme activity under salt stress has been reported in several plants (**Eraslan et al., 2007**; **Bernstein et al., 2010)**.

Applying salinity treatments decreased N, P, K, Ca and Mg contents however Na and Cl were increased. Decreasing N under salinity treatment has been previously reported (**Nazar et al., 2011**; **Tarighaleslami et al., 2012).** Moreover, reduction of P uptake in saline soils was attributed to precipitation of $H_2PO_4$with Ca^{2+} ions in soil and of K and Ca to a competition with Na (**Marschner, 1995**). The reduction of K percentage could be explained through the competition exists between Na^+ and K^+ leading to a reduced level of internal K^+ at high external NaCl concentration (**Botella et al., 1997**). Increasing Na and Cl absorption under salinity in this study is agreeing with **Turan et al. (2007).** Moreover, the accumulation of NaCl disturbed the homeostasis not only Na^{+1} and Cl^{-1} but also of essential cations such as K^{+1} and Ca^{2+} (**Roussos et al., 2007**) and hence a decrease in K^{+1} and Ca^{2+} in rose leaves was observed. In a recent study of **Caia et al.** (**2014**) they concluded that salinity treatment enhances the accumulation of leaf $Na^+$and Cl^-ions, thereby reducing plant growth rate and hence minimizing the ion uptake by the roots and ion accumulation in the shoots are important mechanisms of salt tolerance.

Data obtained in this study showed that treatment with GA_3 had beneficial effects on vegetative growth as well as

physiological and chemical parameters investigated. Moreover, the negative effects of salinity concerning ion homeostasis in rose under saline conditions were alleviated. The vegetative growth promotion of rose plants by GA_3 treatment could be explained through the role of GA_3 in leaf expansion and stem elongation (**Magome et al., 2004**). The reduction in plant growth at different salinity levels was occurred without GA_3 treatment, while under GA_3 treatments the growth characters were enhanced. These results are in agreement with **Misratia et al. (2013)** who mentioned that GA_3 in salt stressed plants showed an increased photosynthetic capacity a vital factor for higher dry matter synthesis. Improving leaf area, chlorophyll content and TSS by GA_3 treatment may be achieved through osmoregulation which in turn increased RWC using the organic solutes (saccharides and proteins), which in turn increased the photosynthetic area of rose plant. Otherwise, applying GA_3 under salinity treatment was found to restore normal chlorophyll levels (**Shaddad et al., 2013**).

Salinity alleviation by GA_3 may occur through its effect on proline metabolism via regulating N or Ca accumulation (**Iqbal et al., 2014)** as our data indicated. Positive interactions between GA_3 and proline have been reported in the literature. **Tuna et al. (2008)** reported that foliar application of GA_3 increased proline content which counteracted some of the adverse effects of salinity by maintaining membrane permeability and increasing macro and micronutrient levels. This enhanced accumulation of proline may represent a major biochemical adaptation in plants osmotic adjustment (**Khan et al., 2010**). GA_3 also increased the antioxidant enzyme activates which may consider as a mechanism for salinity alleviation. GA_3 that primarily affect cell enlargement and growth must also coordinately interact with ABA under stress and possibly other stress metabolites, including antioxidants and ROS scavengers (**Achard et al., 2006**). Foliar application of GA_3 led to increase of Ca^{2+} concentration and induce maintenance of K^+, hence altered ion homeostasis as the obtained data showed. GA_3 may increase Ca^{2+} by increasing the influx of Ca^{2+}at the plasma membrane **(Gilroy and Jones, 1992)**. Similar trend has been reported (**Iqbal and Ashraf, 2010**). Several reports have indicated that GA_3 application on crops produced some benefit in alleviating the adverse effects of salt stress (**Chakrabarti and Mukherji, 2004).**

As a conclusion, salinity treatments negatively affected the growth of rose plants. Under salinity treatments RWC, chlorophyll and leaf area were decreased. However, stomatal density, TSS, proline, membrane permeability and antioxidant enzyme activities were increased. On the other hand, GA_3 treatments alleviated the negative effects of salinity on the growth and physiological and biochemical parameters previously mentioned. GA_3 treatment increased proline content and antioxidant enzymes activities (SOD, CAT and POD) as well as prevented ion homeostasis which may consider possible mechanisms for salinity tolerance in rose.

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